

# Analytical Method Development and Validation

## **CBDA Grant Study Goal:**

Develop and validate sample extraction and analysis procedures that give accurate and reproducible results for environmentally relevant concentrations of pyrethroid insecticides (home and commercial use)

# Analytical Method Development and Validation

## Methods

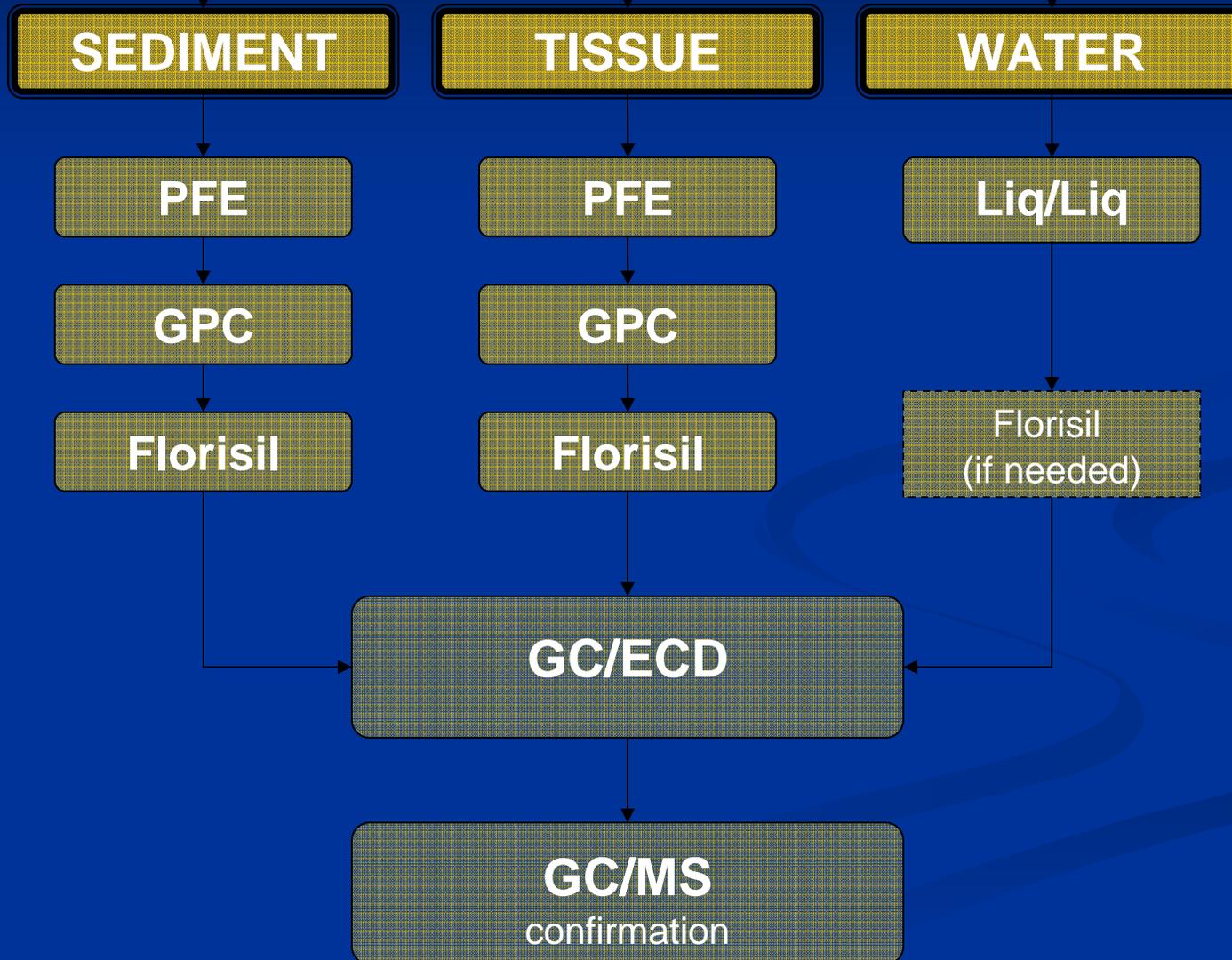
- Compatible with GC and HPLC analyses
- Applicable to water, colloids, sediment and biota
- Applicable at environmentally relevant concentrations
- Amenable to automation for high sample throughput
- Cost effective

# Analytical Method Development and Validation

## Development of Analytical Methods

- Optimization (sensitivity and selectivity) of instrument methods used for analysis and confirmation (GC-ECD, GC-MS, LC-MS, GC-MS/MS and LC-MS/MS)
- Isomer, enantiomer and stereoisomer identification and stability (deltamethrin, tralomethrin)
- Evaluation of water extraction methods (liq-liq, SPE, SPME)
- Evaluation of sediment and tissue extraction and matrix separation methods (PFE, GPC, Florisil)

# Methods for Pyrethroid Analysis



# PYRETHROID EXTRACTION AND ANALYSIS METHOD

**Matrices:** Sediment, Tissue and Water

**Analytes:** Bifenthrin, Cyfluthrin, Cypermethrin, Es-fenvalerate,  
Fenpropathrin, Lambda-cyhalothrin and Permethrin

## Method Summary

**Sediment and Tissue:** ASE - GPC – Florisil

**Water:** Liquid/liquid or Solid Phase Extraction  
– Florisil (*if needed*)

**Analysis:** High resolution GC/ECD with dual columns

Standards obtained from ChemService

Second source obtained from AccuStd

**Reporting Limits:** 0.002-0.012 µg/L (water)

1-10 ng/g dry wt (sediment)

2-6 ng/g fresh wt (tissue)

**Confirmation by GC/MS**

# Water Sample Extraction

## Liquid-liquid Extraction

Sample Prep: 1 liter unfiltered water sample  
2 liter separatory funnel  
Surrogate/Spike solution

Solvent: Methylene chloride (DCM), 120 mL (2x)

Concentrate and solvent exchange:  
Evaporate using K-D and  $\mu$ K-D (75-80°C),  
Exchange solvent with petroleum ether  
(~ 50 mL, 3x)

Reconstitute: 2 mL isooctane

Analysis: GC/ECD

# PYRETHROIDS IN AMERICAN RIVER WATER

Method Validation: Spike Level 2-10 ng/L

Pyrethroids by GC/ECD	Average % Recovery	Average Amount Recovered	Standard Deviation Amount Recovered	MDL=t*Stdev (ng/L)	RL (ng/L)
Bifenthrin	88.1	1.76	0.31	0.98	2.00
Cyfluthrin	103	4.13	2.19	6.88	12.0
Cypermethrin	101	4.04	1.40	4.39	8.00
Deltamethrin	106	2.12	0.51	1.61	4.00
Es/Fenvalerate	101	2.02	0.55	1.73	4.00
Lambda-cyhalothrin	96.2	1.93	0.51	1.61	4.00
Permethrin	102	6.12	0.69	2.17	4.00

# Sediment/Tissue Sample Extraction

## Pressurized Fluid Extraction

Instrument: Automated Dionex ASE 200

ASE Conditions

Solvent: Acetone/Dichloromethane (DCM) 50/50

Program: 100°C, 1500 psi

Sample Prep: 10 g homogenized sediment/tissue

7 g Hydromatrix®

33 mL pre-rinsed extractor cell

Surrogate/spike solution

Extract Collection: Two 60 mL VOA vials per sample

Evaporate and solvent exchange: K-D and  $\mu$ K-D (75-80°C),

Exchange solvent with petroleum ether (~ 50 mL, 3x)

# Gel Permeation Chromatography

Instrument: J2 Scientific AccuPrep 170, Autoinject 110

## GPC Conditions

Solvent: Dichloromethane (DCM)

Column: 60 x 2.5 cm

Column Packing: 65 g BioBeads, 200-400 mesh

Flow: 5 mL/min

Program: Dump-19 min, Collect-24 min, Rinse-10 min

Sample size: 10 mL DCM extract, 5 mL sample loop

Evaporate and solvent exchange: K-D and  $\mu$ K-D (75°C),  
Exchange solvent with petroleum ether (~ 50 mL, 3 times)

# Florisil Column Fractionation

Column Prep: 25 g Preactivated Florisil (130°C, 24 hours) embedded between sodium sulfate with glass wool plug, 11 mm x 300 mm glass column

## Reagents:

0 % ethyl ether in petroleum ether (Fraction 1)

6 % ethyl ether in petroleum ether (Fraction 2)

15 % ethyl ether in petroleum ether (Fraction 3)

# Distribution of Pyrethroids Among Florisil Fractions

(0%) Fraction 1

Do Not Collect  
(PCBs and other  
pesticides)

(6%) Fraction 2

Bifenthrin  
Permethrin

(15%) Fraction 3

Cyfluthrin  
Cypermethrin  
Es-fenvalerate  
Lambda-cyhalothrin  
other pyrethroids

# PYRETHROIDS IN AMERICAN RIVER SEDIMENT

Method Validation: Spike Level 5-20 ng/g

Pyrethroids by GC/ECD	Average % Recovery	Average Amount Recovered	STDEV (n=8) Amount Recovered	MDL=t*Stdev (ng/g)	RL (ng/g)
Bifenthrin	106	5.30	0.14	0.43	1.00
Cyfluthrin	108	10.4	0.85	2.55	4.00
Cypermethrin	108	11.1	0.81	2.44	4.00
Deltamethrin	62.0	3.10	0.69	2.08	4.00
Es/Fenvalerate	107	5.39	0.27	0.80	4.00
Lambda-cyhalothrin	104	5.17	0.38	1.15	4.00
Permethrin	99	16.2	1.27	3.80	8.00

# Round Robin Sediment Results

## SAMPLE 1 RESULTS IN TRIPLICATE

Sample Identification	GC/ECD			Average	STDev
	SED PY 005	SED PY 005	SED PY 005	SED PY 005	SED PY 005
Pyrethroids by GC/ECD	dry wt ppb (ng/g)	dry wt ppb (ng/g)	dry wt ppb (ng/g)	dry wt ppb (ng/g)	
Bifenthrin	11.9	12.3	15.8	13.3	2.19
Cyfluthrin	2.54	1.53	2.76	2.27	0.66
Cypermethrin	3.23	1.63	3.95	2.93	1.19
Delta/Tralo-methrin	ND	ND	ND	ND	NA
Esfen/Fen-valerate	12.2	11.8	12.0	12.0	0.18
<b>Fenpropathrin*</b>	3.75	4.55	4.29	4.20	0.41
Lambda-Cyhalothrin	3.80	3.74	3.63	3.72	0.08
Permethrin	34.2	36.2	38.6	36.3	2.24
<b>Surrogate (% Recovery)</b>					
Dibromooctafluorobiphenyl	79.2	78.6	106	88.1	NA
Dibromochlorendated	82.6	78.4	79.6	80.2	NA

\*Estimated MDL

# Round Robin Sediment Results

## SAMPLE 2 RESULTS IN TRIPLICATE

Sample Identification	GC/ECD			Average	STDev
	SED PY 006	SED PY 006	SED PY 006	SED PY 006	SED PY 006
Pyrethroids by GC/ECD	dry wt ppb (ng/g)	dry wt ppb (ng/g)	dry wt ppb (ng/g)	dry wt ppb (ng/g)	
Bifenthrin	15.0	13.3	14.0	14.1	0.86
Cyfluthrin	1.48	1.25	0.66	1.13	0.42
Cypermethrin	1.04	1.56	2.86	1.82	0.94
Delta/Tralo-methrin	ND	ND	ND	ND	NA
Esfen/Fen-valerate	11.2	11.7	12.3	11.8	0.55
<b>Fenpropathrin*</b>	6.62	6.03	6.56	6.40	0.33
Lambda-Cyhalothrin	4.01	3.48	4.51	4.00	0.52
Permethrin	36.7	37.9	42.6	39.1	3.13
<b>Surrogate (% Recovery)</b>					
Dibromooctafluorobiphenyl	77.4	89.6	94.0	87.0	NA
Dibromochlorendated	81.6	83.6	82.2	82.5	NA

\*Estimated MDL

# Round Robin Sediment Results

## QC RESULTS

Sample Identification	Method Blank	American River Sediment LCS	American River Sediment LCSD	SED PY 005 MS
Pyrethroids by GC/ECD	dry wt ppb (ng/g)	% Recovery	% Recovery	% Recovery
Bifenthrin	ND	102	87.8	81.7
Cyfluthrin	ND	75.7	70.2	96.6
Cypermethrin	ND	80.6	71.2	101
Delta/Tralo-methrin	ND	35.7	ND	77.6
Esfen/Fen-valerate	ND	73.1	66.3	112
Fenpropathrin*	ND	76.8	83.7	92.0
Lambda-Cyhalothrin	ND	79.6	70.3	105
Permethrin	ND	95.3	104	104
<b>Surrogate (% Recovery)</b>				
Dibromooctafluorobiphenyl	89.4	88.0	81.2	109
Dibromochlorendated	71.0	72.4	72.8	84.0

\*Estimated MDL

# Round Robin Sediment Results

## GC/ECD vs GC/MS/MS (TSQ)

Sample Identification	SED PY 005 dry wt. ppb (ng/g)		SED PY 005 dry wt. ppb (ng/g)		SED PY 005 dry wt. ppb (ng/g)	
	GC/ECD	GC/MS/MS (TSQ)	GC/ECD	GC/MS/MS (TSQ)	GC/ECD	GC/MS/MS (TSQ)
<b>Pyrethroid Pesticides</b>						
Bifenthrin	11.9	10.2	12.3	13.7	15.8	17.4
Cyfluthrin	2.54	1.53	1.53	2.10	2.76	1.91
Cypermethrin	3.23	2.56	1.63	3.07	3.95	6.91
Delta/Tralo-methrin	ND	ND	ND	ND	ND	ND
Esfen/Fen-valerate	12.2	14.4	11.8	16.0	12.0	17.4
<b>Fenpropathrin*</b>	3.75	8.05	4.55	10.40	4.29	9.69
Lambda-Cyhalothrin	3.80	3.20	3.74	4.17	3.63	4.86
Permethrin	34.2	43.2	36.2	51.1	38.6	67.6

\*Estimated MDL

# Round Robin Sediment Results

## GC/ECD vs GC/MS/MS (TSQ)

Sample Identification  Pyrethroid Pesticides	SED PY 006 dry wt. ppb (ng/g)		SED PY 006 dry wt. ppb (ng/g)		SED PY 006 dry wt. ppb (ng/g)	
	GC/ECD	GC/MS/MS (TSQ)	GC/ECD	GC/MS/MS (TSQ)	GC/ECD	GC/MS/MS (TSQ)
Bifenthrin	15.0	18.7	13.3	17.0	14.0	19.0
Cyfluthrin	1.48	3.14	1.25	1.09	0.66	1.96
Cypermethrin	1.04	2.83	1.56	2.92	2.86	3.51
Delta/Tralo-methrin	ND	ND	ND	ND	ND	ND
Esfen/Fen-valerate	11.2	16.1	11.7	15.6	12.3	18.1
<b>Fenpropathrin*</b>	6.62	8.20	6.03	8.28	6.56	12.2
Lambda-Cyhalothrin	4.01	4.18	3.48	4.10	4.51	4.64
Permethrin	36.7	73.4	37.9	68.7	42.6	83.0

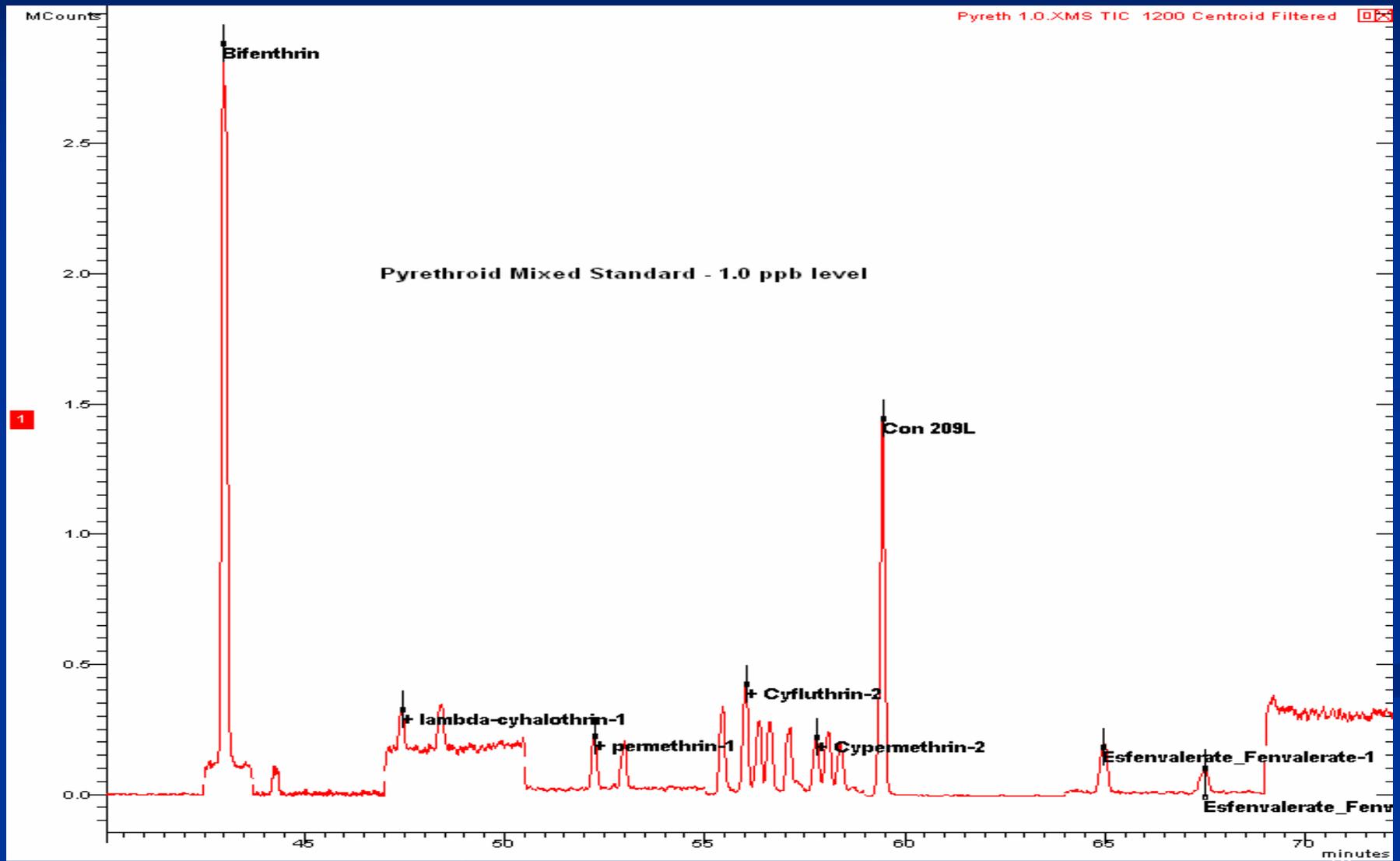
\*Estimated MDL

# Retention times, Q1-Q3 Transitions and Collision Energy

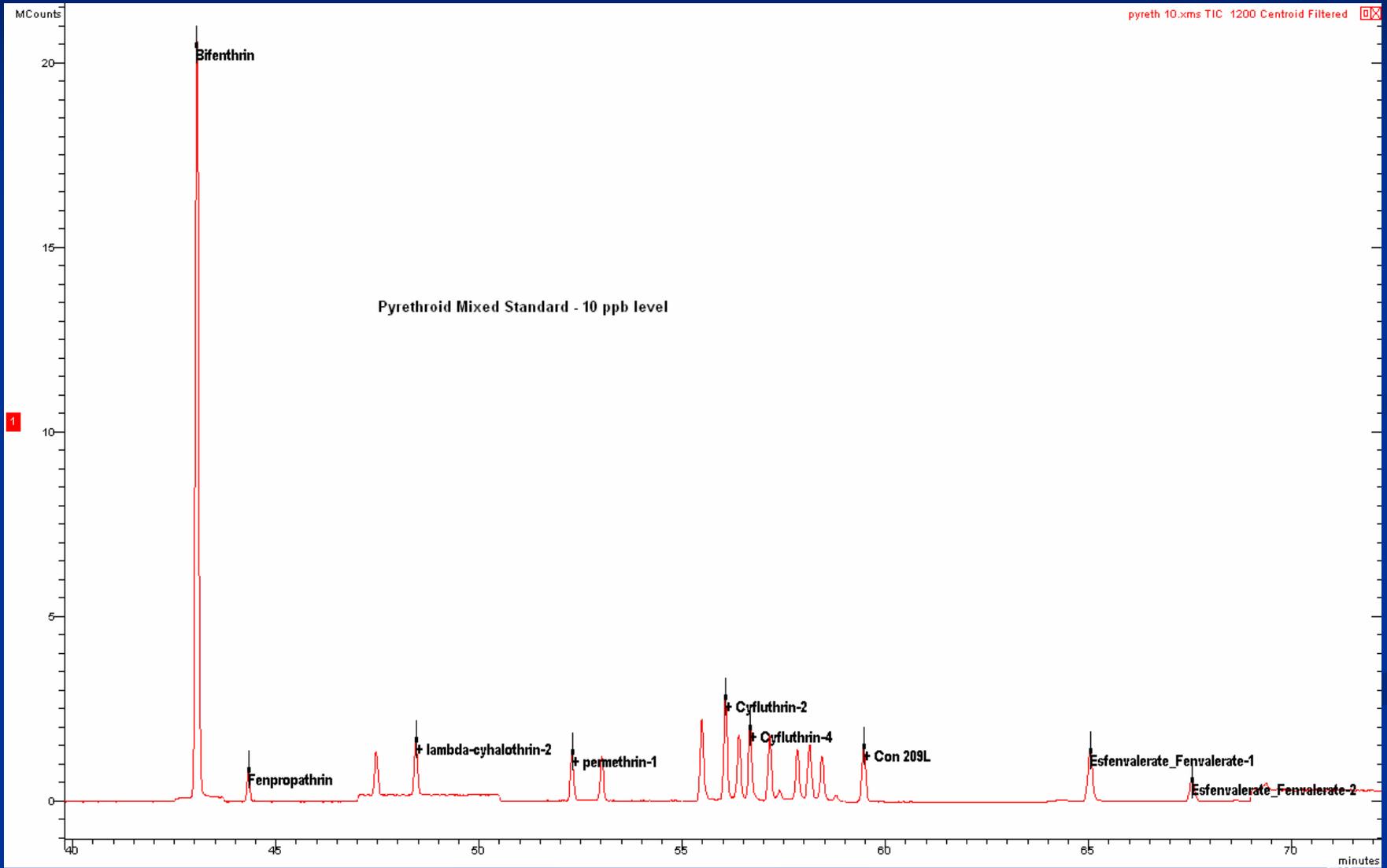
RT	Analyte	Q1	Q3	Collision Energy (-volts)
43.916	Bifenthrin	181	166	15
48.294	Lambda-cyhalothrin-1	181	152	25
49.296	Lambda-cyhalothrin-2	181	152	25
53.156	Permethrin-1	183	153+163	10
53.909	Permethrin-2	183	153+163	10
56.384	Cyfluthrin-1	163	127	10
57.021	Cyfluthrin-2	163	127	10
57.327	Cyfluthrin-3	163	127	10
57.622	Cyfluthrin-4	163	127	10
58.164	Cypermethrin-1	163	127	10
58.876	Cypermethrin-2	163	127	10
59.191	Cypermethrin-3	163	127	10
59.510	Cypermethrin-4	163	127	10
64.970	Es/fenvalerate	167	125	15
66.453	Es/fenvalerate	167	125	15
71.039	Deltamethrin	181	152	20

# Pyrethroid Mixed Standard MS/MS

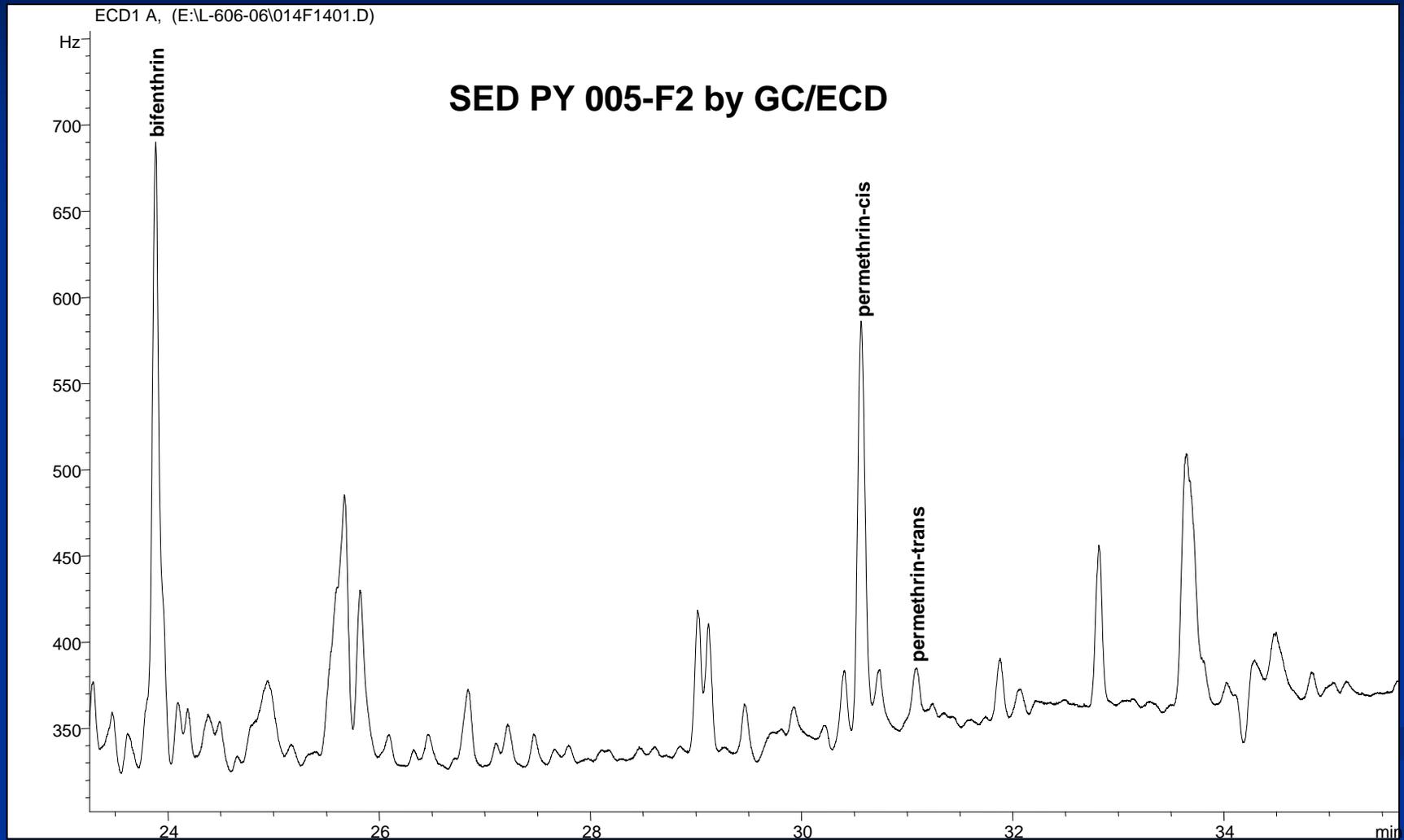
## 1.0 ppb



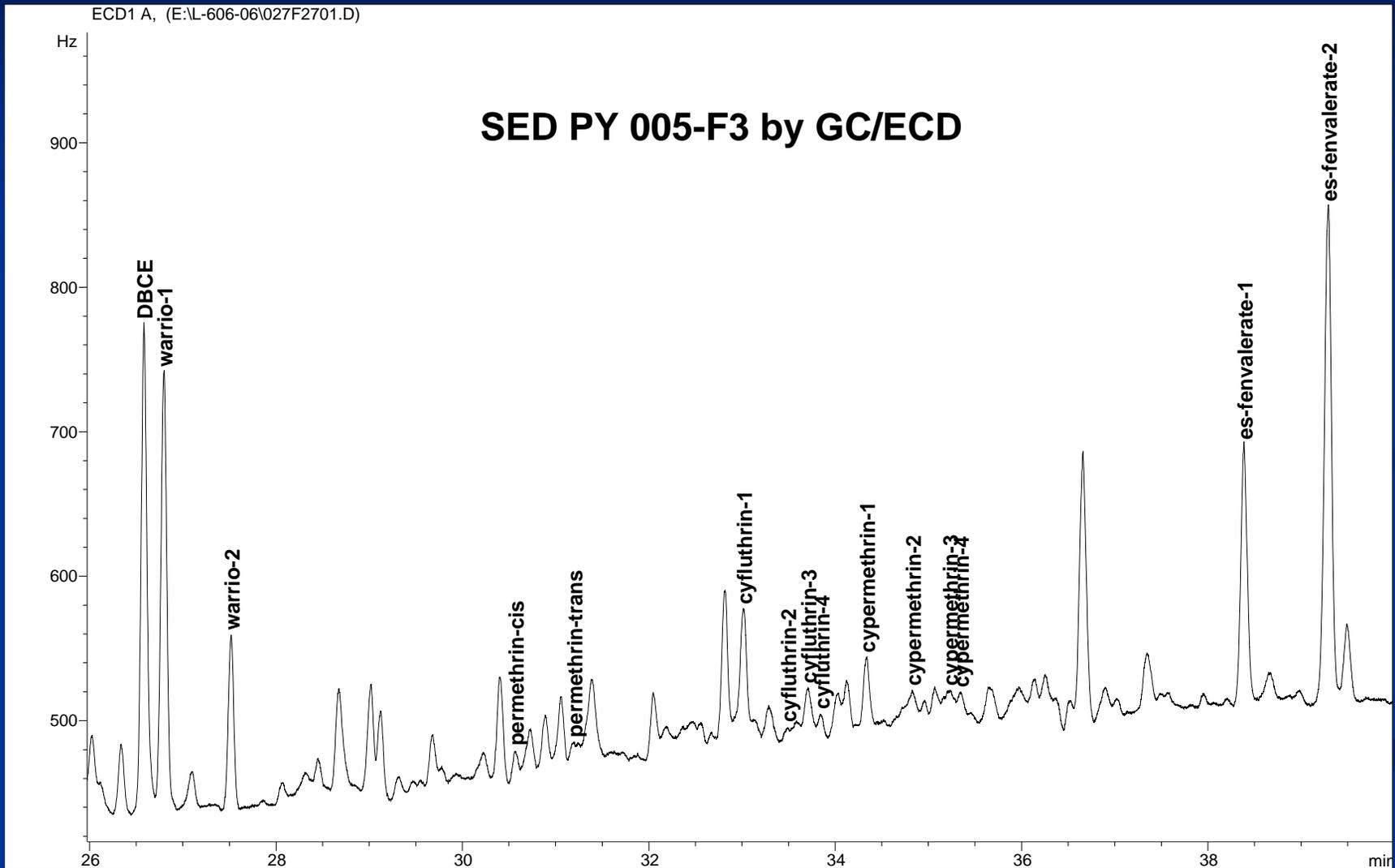
# Pyrethroid Mixed Standard 10 ppb



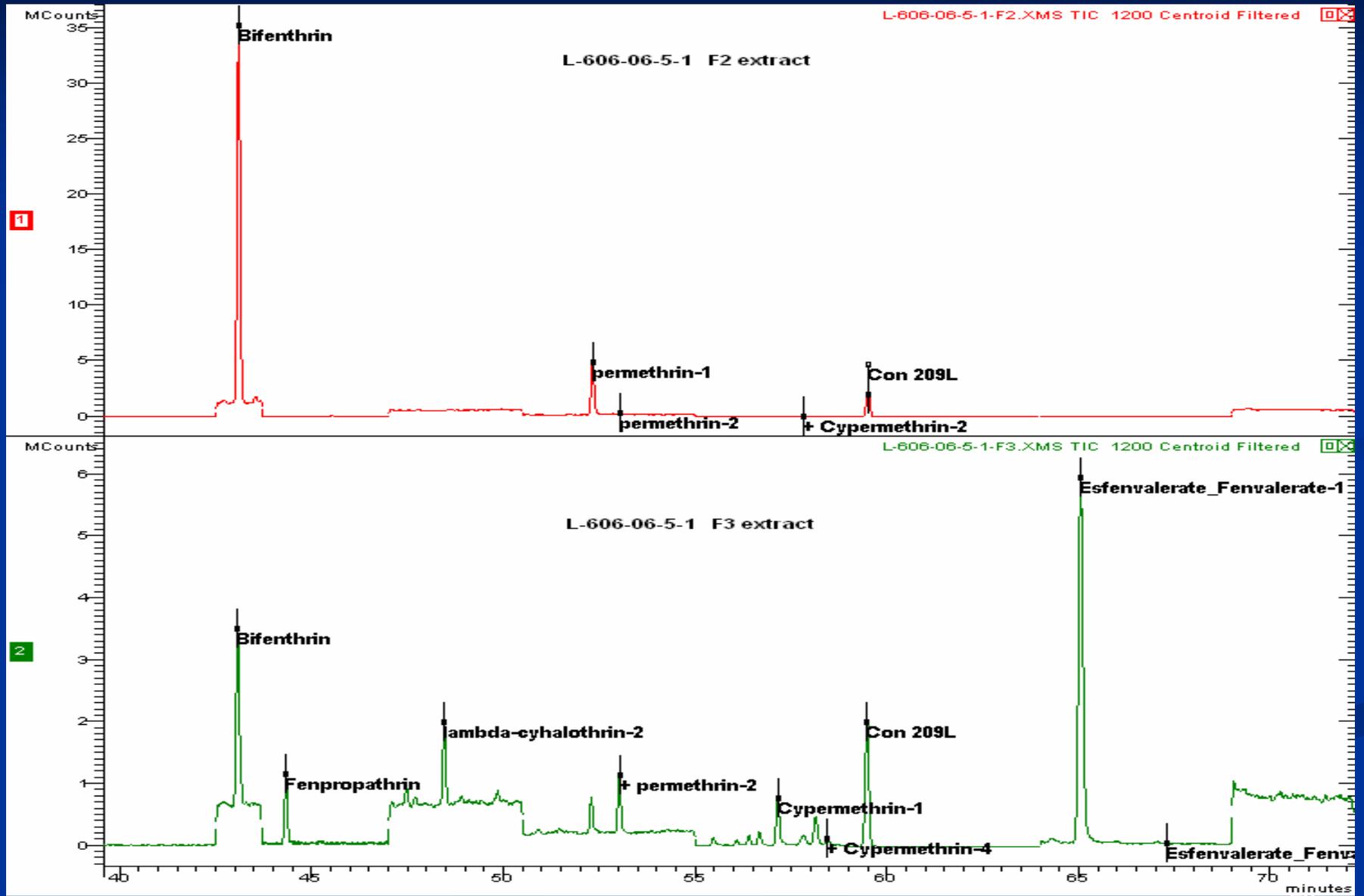
# Round Robin Sediment Extract



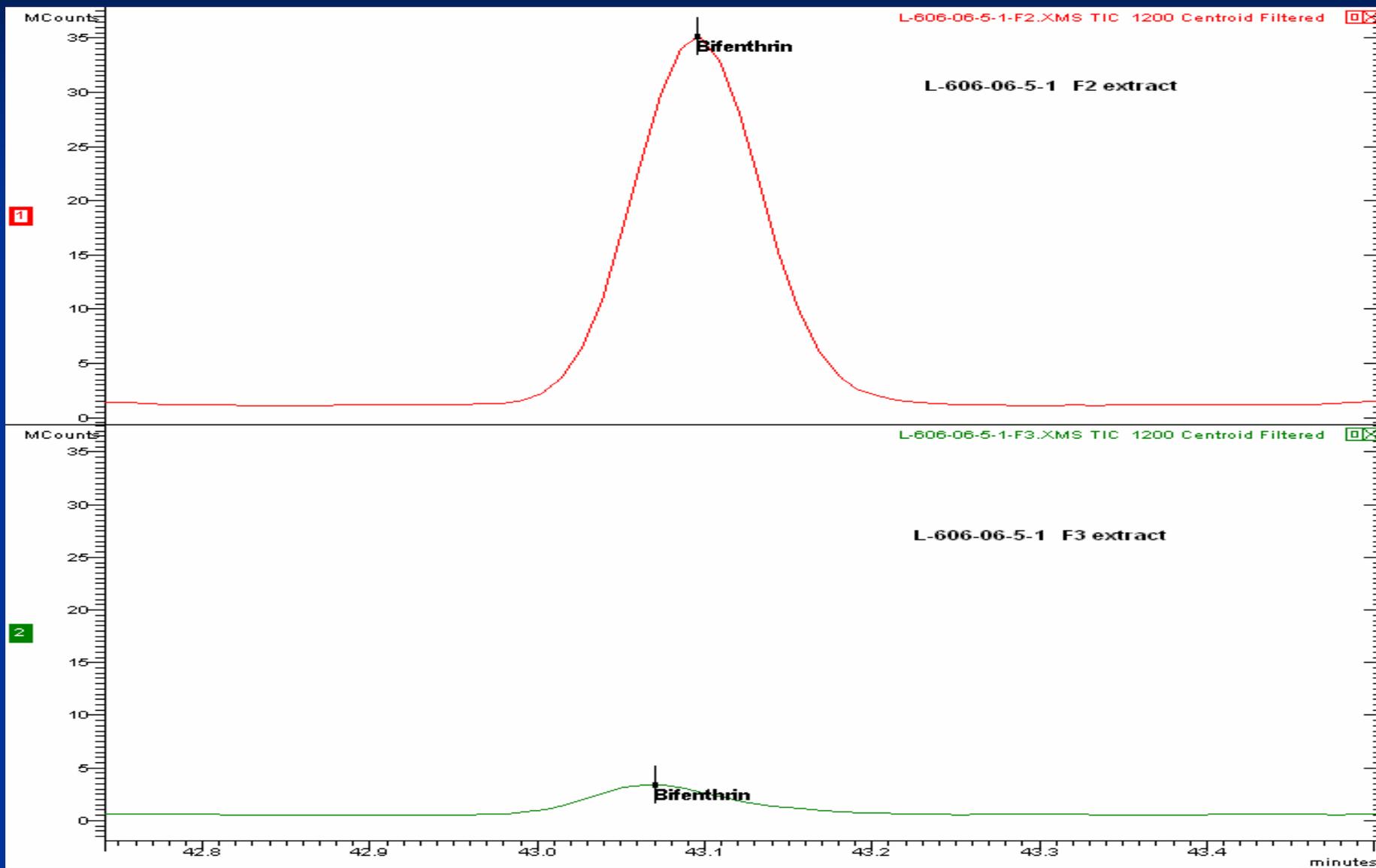
# Round Robin Sediment Extract



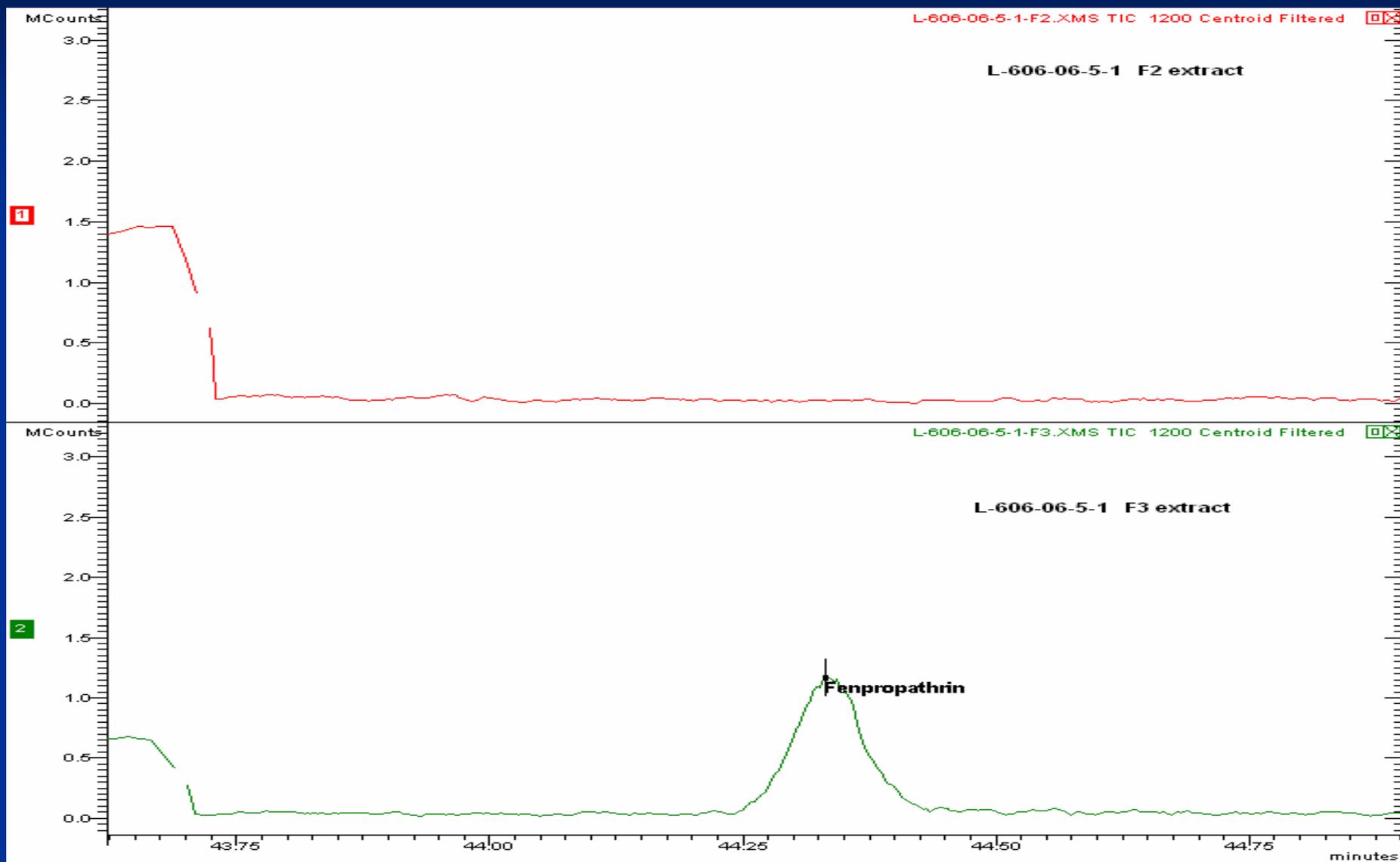
# Round Robin Sediment Extract



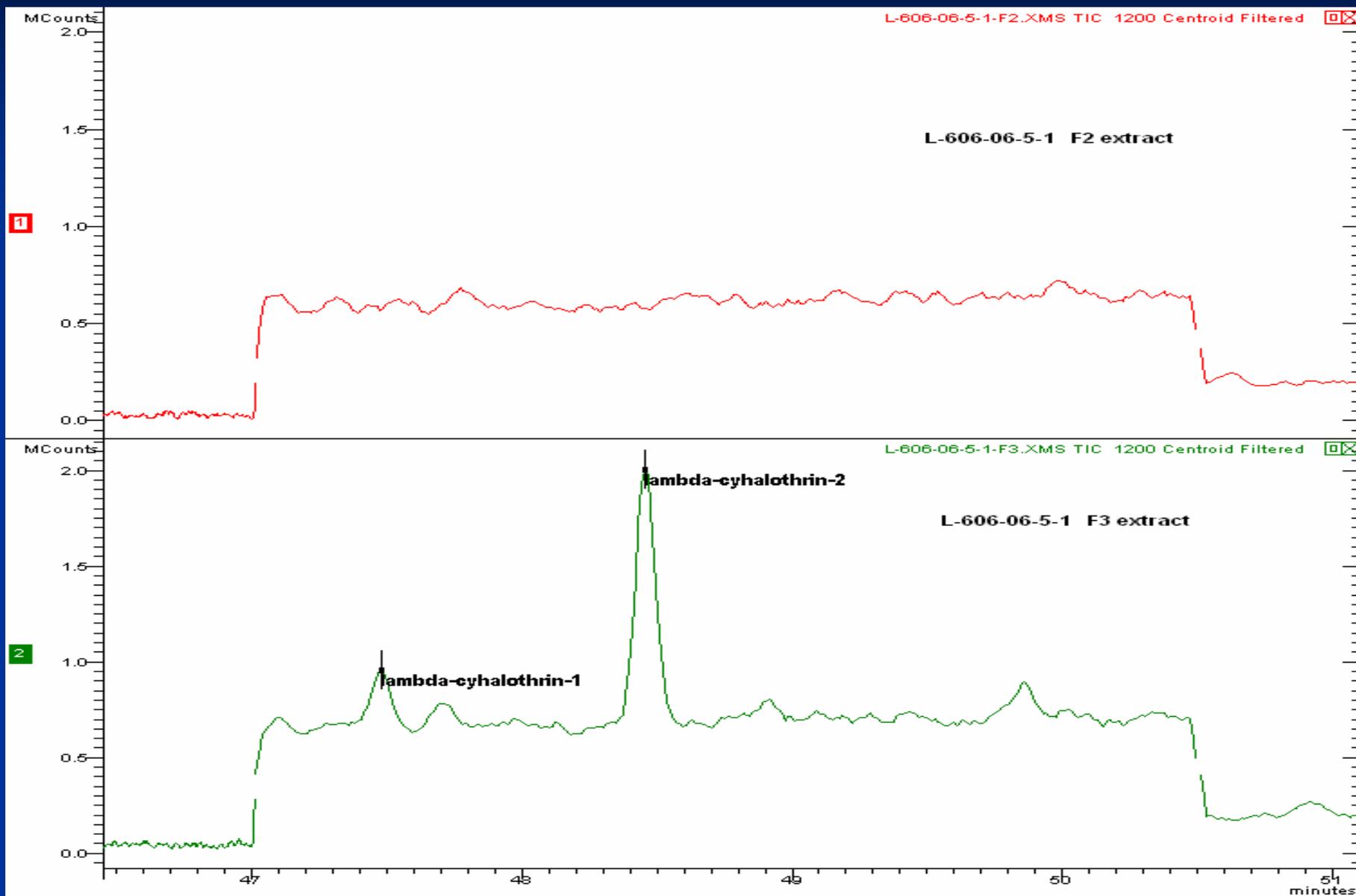
# Round Robin Sediment Extract



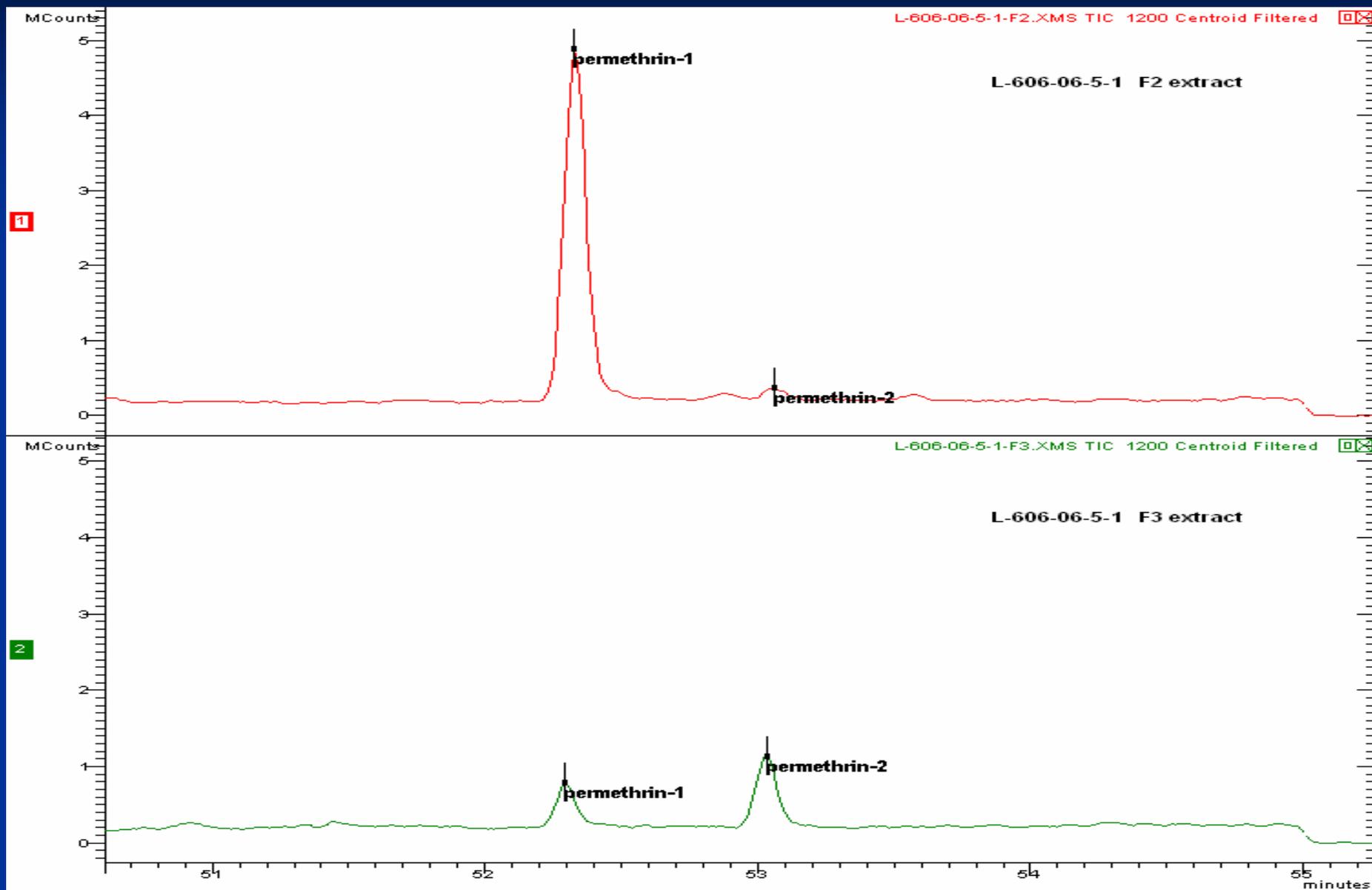
# Round Robin Sediment Extract



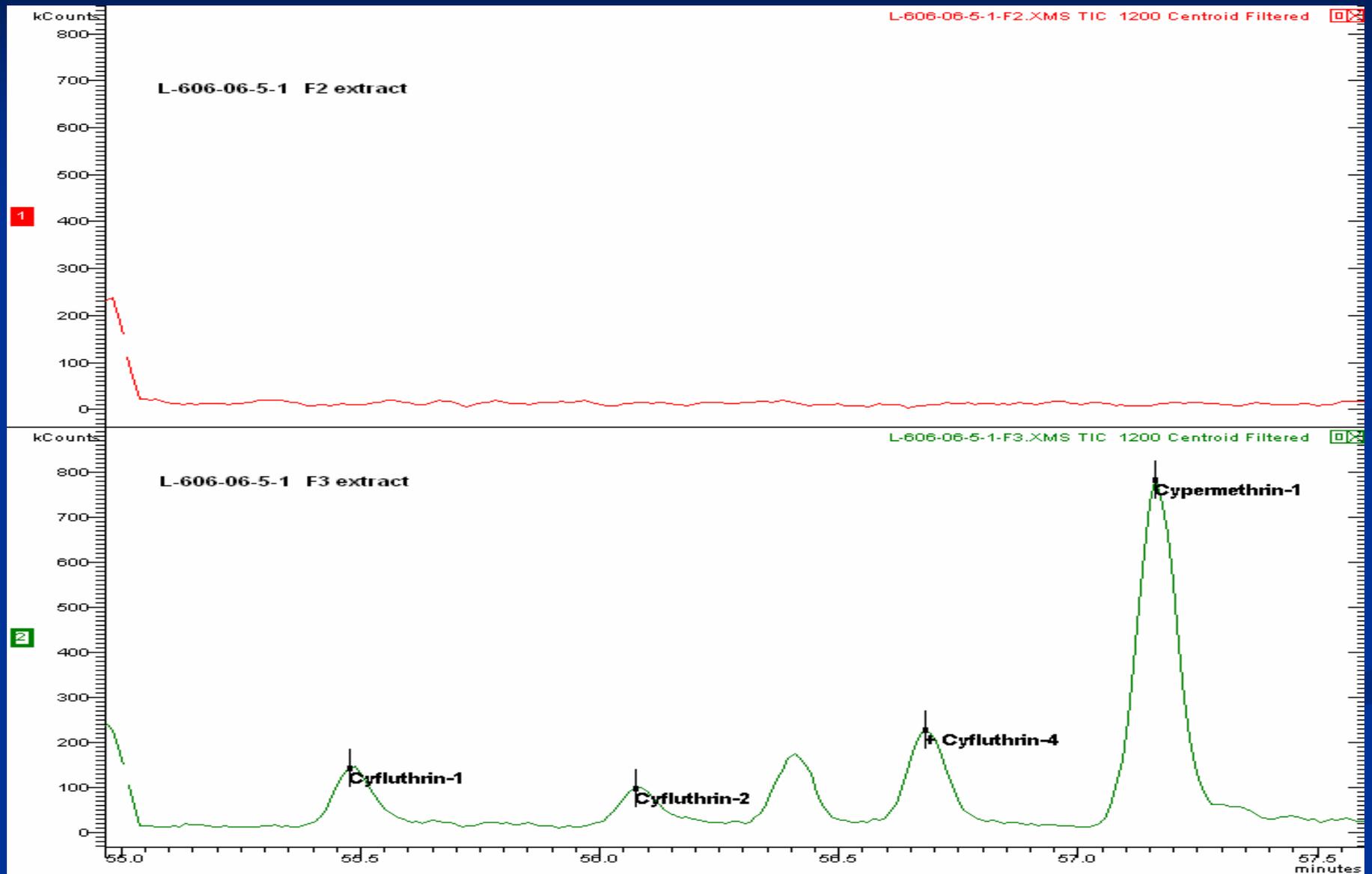
# Round Robin Sediment Extract



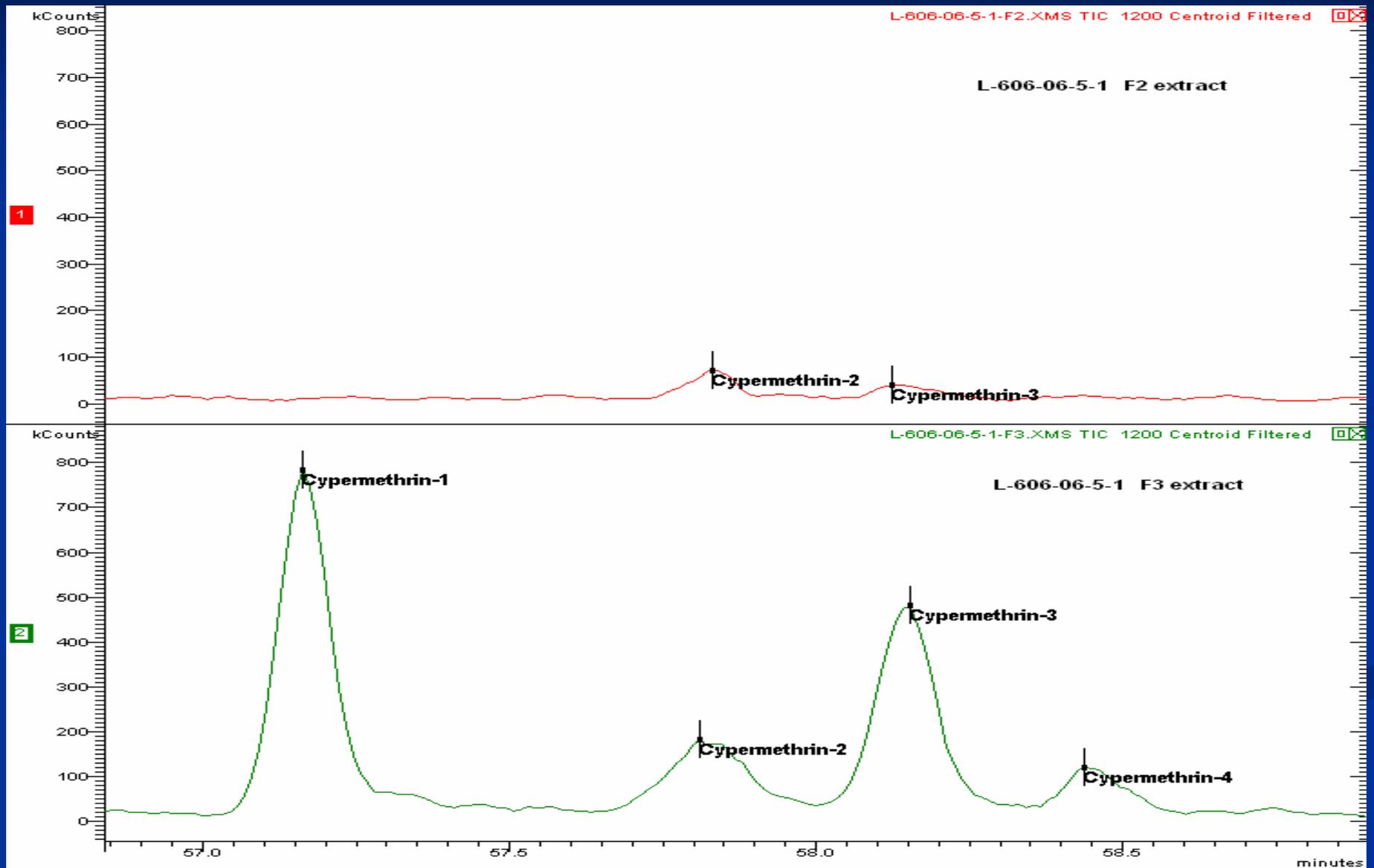
# Round Robin Sediment Extract



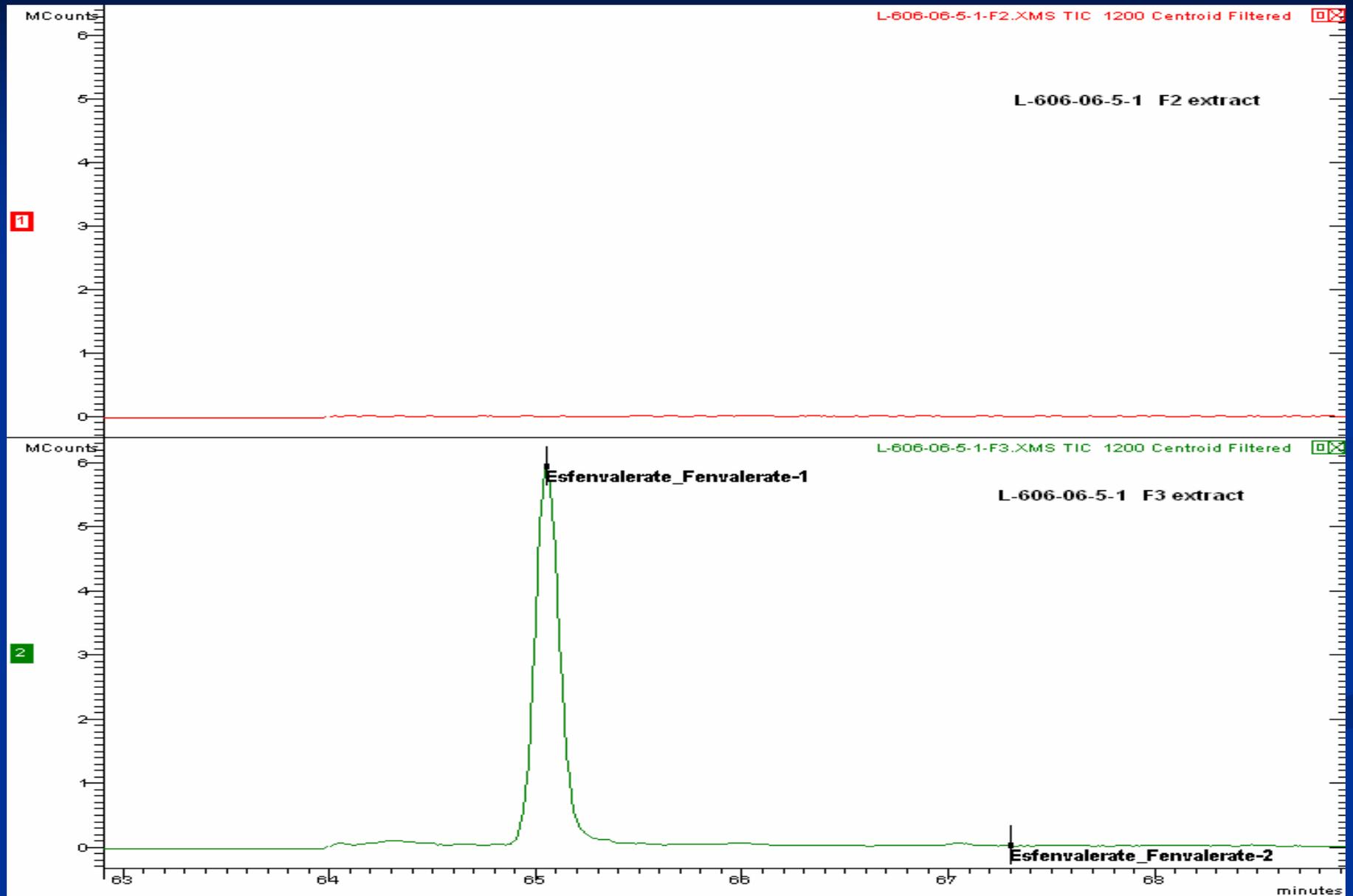
# Round Robin Sediment Extract



# Round Robin Sediment Extract



# Round Robin Sediment Extract



# PYRETHROID TISSUE VALIDATION

American River Hatchery Trout, Spike Level 4-16 ng/g

Pyrethroid Pesticides	AVERAGE % Recovery	AVERAGE Amount Recovered	STD DEV (n=7) Amount Recovered	MDL=t*Stdev Fresh Wt. ppb (ng/g)	RL Fresh Wt. ppb (ng/g)
Bifenthrin	88.1	3.52	0.21	0.65	2.00
Cyfluthrin	98.7	15.8	0.86	2.72	6.00
Cyhalothrin-Lambda	89.1	3.56	0.47	1.49	4.00
Cypermethrin	74.3	5.94	0.53	1.660	4.00
Deltamethrin	41.8	1.67	0.19	0.60	2.00
Esfenvalerate	52.8	2.11	0.53	1.68	4.00
Fenpropathrin*	85.0	3.40	0.43	1.34	4.00
Permethrin	97.2	11.7	0.71	2.23	6.00

\*Estimated MDL

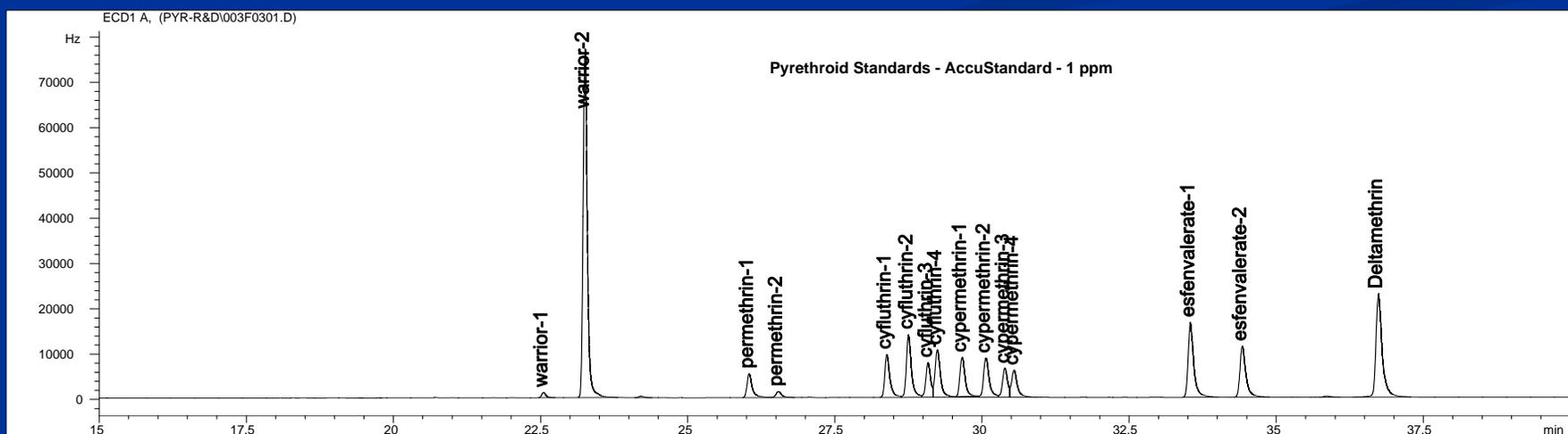
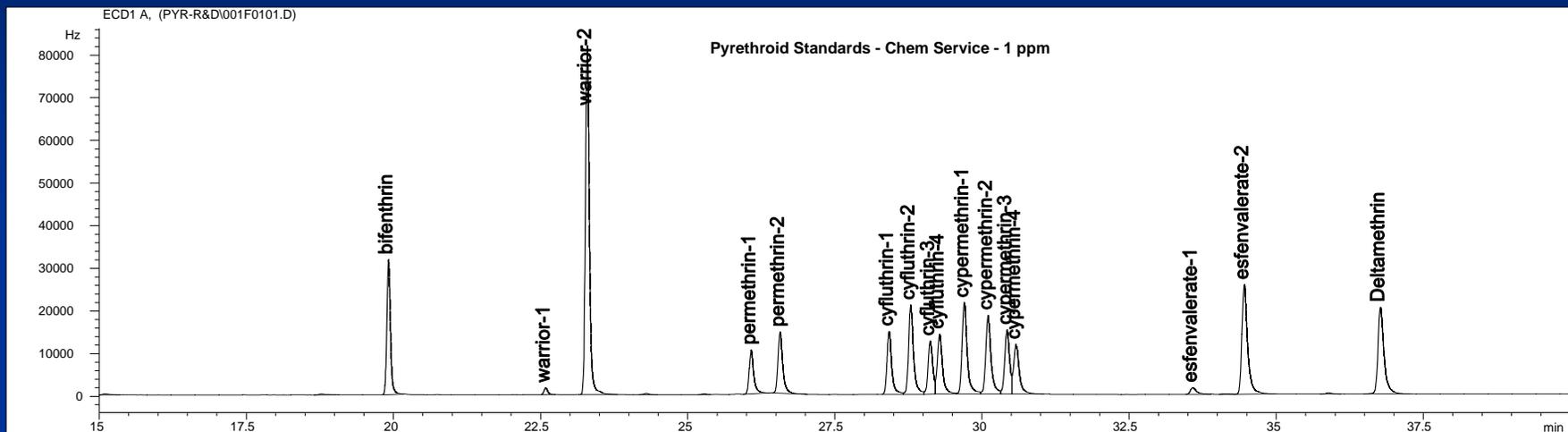
# PYRETHROID METHOD DETECTION LIMITS AND REPORTING LIMITS FOR WATER, SEDIMENT AND TISSUE By GC/ECD

Sample Matrix	Water		Sediment		Tissue	
	MDL	RL	MDL	RL	MDL	RL
Pyrethroid Pesticides	ppb (ug/L)	ppb (ug/L)	Dry wt ppb (ng/g)	Dry wt ppb (ng/g)	Fresh wt ppb (ng/g)	Fresh wt ppb (ng/g)
Bifenthrin	0.001	0.002	0.50	1.00	0.65	2.00
Cyfluthrin	0.006	0.012	2.00	4.00	2.70	6.00
Cypermethrin	0.004	0.008	2.00	4.00	1.70	4.00
Deltamethrin	0.002	0.004	2.00	4.00	0.60	2.00
Es/Fenvalerate	0.002	0.004	2.00	4.00	1.70	4.00
Fenpropathrin*	0.002	0.004	2.00	4.00	1.35	4.00
Lambda-cyhalothrin	0.002	0.004	2.00	4.00	1.50	4.00
Permethrin	0.002	0.004	4.00	8.00	2.20	6.00

\*Estimated MDL

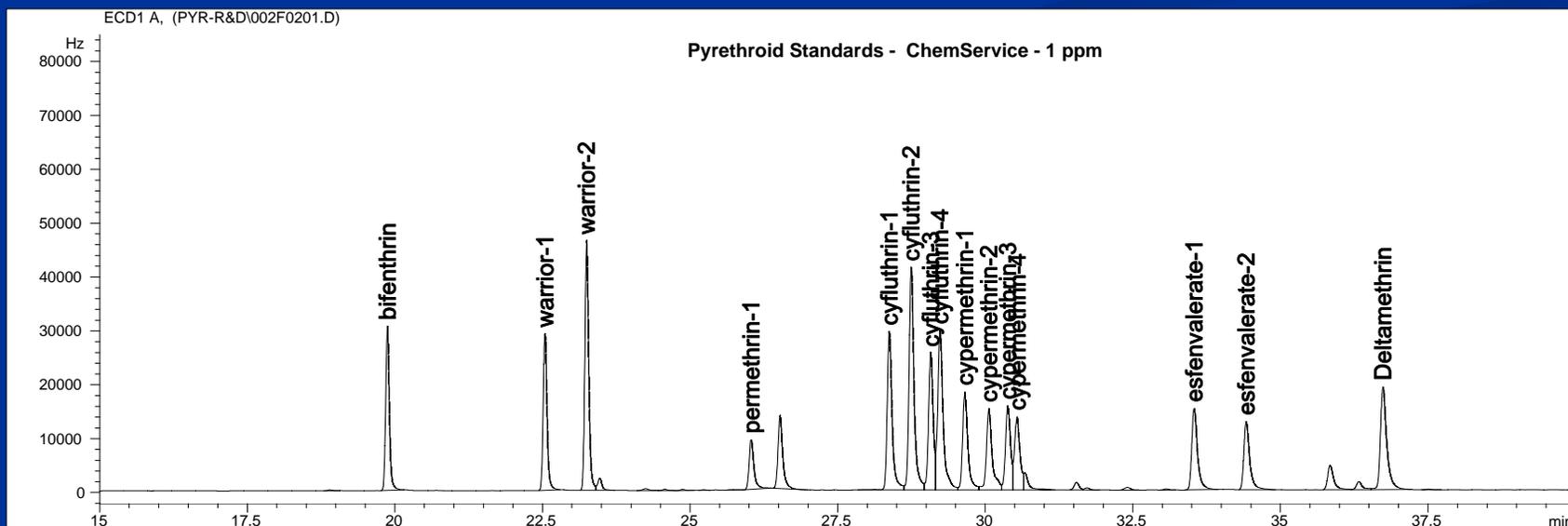
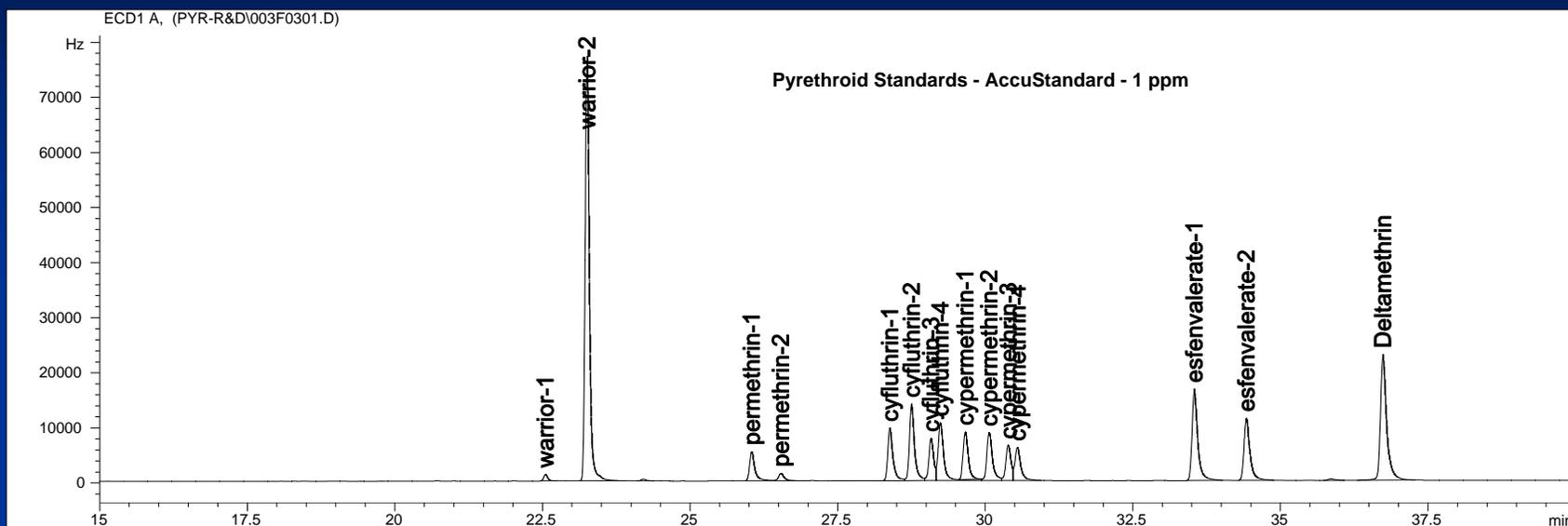
# PYRETHROID STANDARD MATERIAL

## DIFFERENCE IN ISOMER RATIOS BY VENDOR



# PYRETHROID STANDARD MATERIAL

## DIFFERENCE IN ISOMER RATIOS BY VENDOR



# ADDITIONAL PYRETHROID ANALYTES

## Fortified American River Water

Spike Level: 20 ng/L

Extraction Method Pyrethroids by GC/ECD	AR 1	AR 2	AR 3		
	liq/liq	liq/liq	liq/liq	AVERAGE % Recovery	STD DEV
Allethrin	109	98.4	108	105	5.85
Flucythrinate	88.3	83.3	84.3	85.3	2.65
Phenothrin	87.5	88.5	95.5	90.5	4.36
Prallethrin	94.7	86.8	92.6	91.4	4.09
Tetramethin	112	114	116	114	2.00
Tralomethrin	43.5	51.3	56.2	50.3	6.40
Surrogate (%Rec)					
DBOB	90.0	98.3	112	100	11.1

# ADDITIONAL PYRETHROID ANALYTES

## Fortified American River Water

Spike Level: 40 ng/L

Extraction Method Pyrethroids by GC/ECD	AR 1	AR 2	AR 3		
	SPE-Acidified	SPE-Acidified	SPE-Acidified	AVERAGE	STD DEV
	% Recovery	% Recovery	% Recovery	% Recovery	
Allethrin	113	101	105	106	6.11
Flucythrinate	92.5	91.8	88.8	91.0	1.97
Phenothrin	97.2	94.0	97.3	96.2	1.88
Prallethrin	118	107	109	111	5.86
Tetramethin	128	113	119	120	7.55
Tralomethrin	85.6	80.2	77.0	80.9	4.35
Surrogate					
DBOB	95.2	86.1	85.3	88.9	5.50

# ADDITIONAL PYRETHROID ANALYTES

## Fortified American River Sediment

Spike Level: 4 ng/g

Extraction Method Pyrethroids by GC/ECD	AR 1	AR 2	AR 3	AVERAGE % Recovery	STD DEV
	ASE % Recovery	ASE % Recovery	ASE % Recovery		
Allethrin	126	101	110	112	12.7
Flucythrinate	113	105	108	109	4.04
Phenothrin	101	99.2	77.7	92.6	13.0
Prallethrin	122	103	103	109	11.0
Tetramethin	120	106	127	118	10.7
Tralomethrin	93.0	91.6	94.5	93.0	1.45
Surrogate (%Rec)					
DBOB	126	104	100	110	14.0

# SUMMARY

- **Methods developed and validated for water, sediment and tissue**
- **Method of quantitation - total vs. isomers**
- **Method extends to other pyrethroids – allethrin, flucythrinate, phenothrin, prallethrin, tetramethin and tralomethrin**
- **GC/MS/MS (TSQ): Very sensitive – at least 2 – 10 times lower than GC/ECD; best tool to obtain lower reporting limit by reducing background interference**

# FUTURE METHOD DEVELOPMENT

- **Optimize GC/MS/MS analysis by using appropriate internal standards and surrogates**
- **Complete validation of GC/MS/MS-TSQ**
- **Complete validation of GC/MS/MS-Ion Trap**
- **SPME Method Development using MS/MS**
- **Continue LC/MSD investigation**